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Bioprocessing of Ores: Application to Space Resources

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Introduction

The role of microorganisms in the oxidation and leaching of various ores (especially those of copper, iron, and uranium) is well known. (Among the review articles and reference books on this subject are Brierley 1978 and 1982; Brock, Smith, and Madigan 1984; Decker 1986; Ehrlich 1981; Kelly, Norris, and Brierley 1979; Krumbein 1983; Lundgren and Silver 1980; and Torma and Banhegyi 1984.) This role is

increasingly being applied by the mining, metallurgy, and sewage industries in the biobeneficiation of ores and in the bioconcentration of metal ions from natural receiving waters and from waste waters high in toxic metals (Belliveau et al. 1987, Ehrlich and Holmes 1986, Hutchins et al. 1986, Nicolaidis 1987, Olson and Brinckman 1986, Olson and Kelly 1986, Thompson 1986, Torma 1987a and b, Tsezos 1985, Volesky 1987, Woods and Rawlings 1985). See figure 28.

Figure 28

Bacterial Processing of Metal Ores

Although most concepts of processing lunar and asteroidal resources involve chemical reactors and techniques based on industrial chemical processing, it is also possible that innovative techniques might be used to process such resources. Shown here are rod-shaped bacteria leaching metals from ore-bearing rocks through their metabolic activities. Bacteria are already used on Earth to help process copper ores. Advances in genetic engineering may make it possible to design bacteria specifically tailored to aid in the recovery of iron, titanium, magnesium, and aluminum from lunar soil or asteroidal regolith. Biological processing promotes the efficacy of the chemical processes in ore beneficiation (a synergistic effect). Taken from Brierley 1982.



The question of harnessing bacteria for the beneficiation of ores on the Moon, on asteroids, or on Mars has been raised and must be seriously considered in the context of the utilization of space resources. Because of the almost total lack of organic matter on the Moon, it is fortunate that most bacteria known to participate in acid leaching of ores are autotrophic (derive all their carbon from carbon dioxide) as well as chemolithotrophic (derive energy through oxidation of reduced inorganic compounds or elements; e.g., hydrogen sulfide or ferrous ions). Furthermore, they satisfy all of their nutritional needs with inorganic substances, including

certain trace elements known to be present in the Moon's regolith. But the development of biological processes to extract and purify ores on the Moon is severely constrained by the environmental conditions: the lack of elemental oxygen; the limited carbon, nitrogen, and hydrogen; the apparent lack of water; and the extremes of temperature and radiation. Thus, microbial ore processing must be established within a gas-tight enclosure. The enclosure must allow replenishment or augmentation of nutritional needs, retain moisture, maintain a suitable temperature, and protect the cells from radiation. See figure 29.



Figure 29

Enclosure for Microbial Ore Processing

A variety of pressurized enclosures have been proposed for a lunar base. In this concept by Fluor Daniel and Rockwell International, a large hangar-like enclosure functions as a workshop and servicing area. A microbial ore-processing complex could be totally enclosed by such a building. Ore could be introduced through airlocks and processed in a moist atmosphere at an appropriate temperature. Spent ore could be removed through airlocks, and waste products could be recycled. The key point is that the harsh environment at the lunar surface can be suitably modified to provide an optimum environment for ore processing.

Artist: Renato Moncini

Various kinds of interactions between microorganisms and metals are known: (1) beneficial as well as toxic effects of metals on metabolism, (2) oxidation-reduction reactions, (3) solubilization of metals through acids produced by microbial growth, (4) precipitation of metals by pH increases, (5) conjugation of metals and organic compounds, (6) metabolic transformation of metals, and (7) accumulation of metals either on the inside or on the outside of cells. In this paper I will consider the processes that are particularly important to the technology of metal recovery. Some of them may have application in the space environment. However, essentially no research has been done with this application in mind.

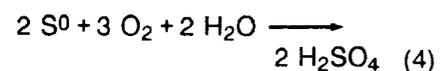
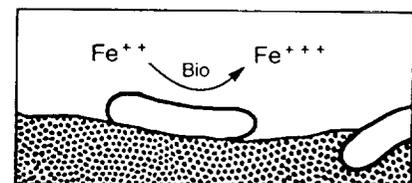
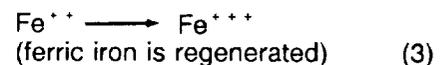
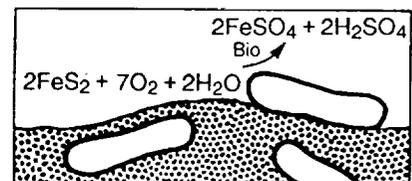
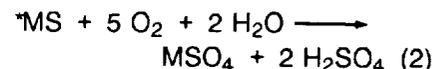
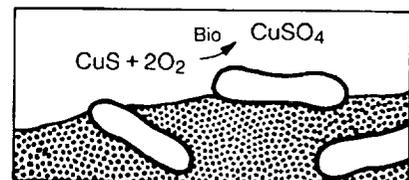
Acid Leaching of Ores

Acid leaching is a hydrometallurgical process resulting in the solubilization of ore minerals through chemical and biological oxidations and reductions of sulfur, iron, and certain other metals.

The Chemistry

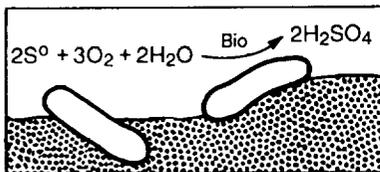
The chemistry of the process is complex and greatly affected by pH, oxidation-reduction potential, dissolved oxygen, and temperature. (Some of the authorities describing this chemistry are Hutchins et al.

1986; Kelly, Norris, and Brierley 1979; Lundgren, Valkova-Valchanova, and Reed 1986; Torma and Banhegyi 1984; and Torma 1987a.) For example, bacteria can catalyze and drive the following oxidations:

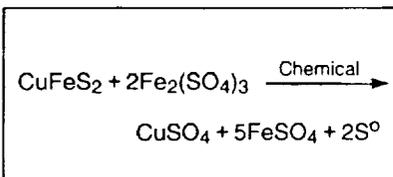
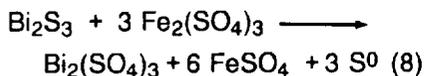
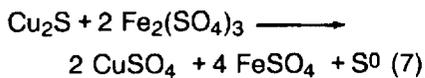
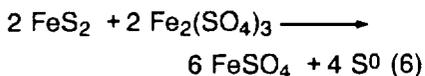


*M = Fe, Cu, Mo, Sb, Pb, Ag, Co, Ni, Cu-Fe, As-Fe, Ni-Fe, Zn-Fe, Cu-Se, etc.

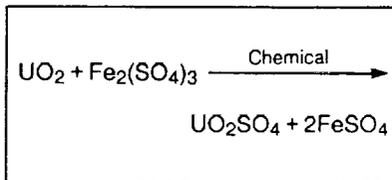
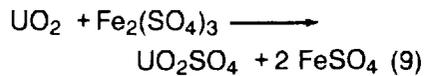
(Thionate, tetrathionate, thiosulfate, and sulfite are also oxidized by some bacteria.)



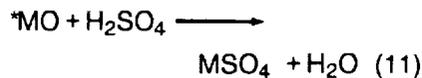
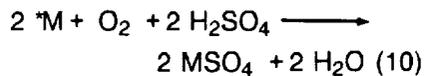
Chemically, ferric sulfate (largely generated through bacterial oxidation of ferric sulfide or ferrous ions) and sulfuric acid (largely generated through bacterial oxidation of ferric and other metal sulfides, elemental sulfur, or hydrogen sulfide) oxidize and solubilize numerous minerals; for example,



Another example of ferric sulfate leaching



Solubilization of uranous oxide



The Bacteriology

The principal bacteria catalyzing reactions 1 through 5 are mixed populations of *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Thiobacillus thiooxidans*, and several other species of acidophilic thiobacilli. There also exist some thermophilic thiobacilli which are facultative autotrophs inasmuch as they can utilize certain organic substrates in the environment. *Thiobacillus ferrooxidans* is unique in that it derives energy from oxidations of sulfur and reduced iron, copper, and tin; it also fixes nitrogen. *Thiobacillus thiooxidans* cannot oxidize iron; rather, it oxidizes sulfur and probably zinc sulfide. *Leptospirillum ferrooxidans* will

oxidize only the soluble form of iron (Fe^{++}), but in conjunction with certain other sulfur-oxidizing thiobacilli it will synergistically oxidize pyrite (FeS_2) as well as chalcopyrite (CuFeS_2).

Another group of bacteria, the genus *Sulfolobus*, which is widely distributed in volcanic vents and thermal springs, is able to oxidize sulfur and iron at temperatures of 80°C , or even a few degrees higher. Sulfolobi are also able to grow in the absence of molecular oxygen provided Mo^{6+} or Fe^{+++} are present to serve as electron acceptors, thereby replacing O_2 as the ultimate electron acceptor. They are facultative heterotrophs and occupy a unique niche in the bacterial kingdom as *Archaeobacteriaceae*, a family possessing a cell membrane composed of a long-chain, ether-linked hydrocarbon monolayer (instead of a phospholipid bilayer) and lacking a peptidoglycan cell wall, which is found in all other bacteria (Kelly and Deming 1988). Whether these and certain other archaeobacteria play a significant role in thermophilic leaching of ores can only be surmised.

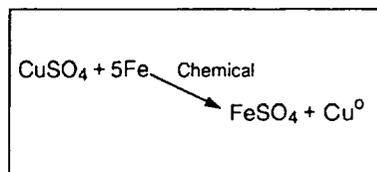
A filamentous, moderately thermophilic, sulfur-oxidizing, autotrophic bacterium, *Thermothrix thiopara*, also flourishes in volcanic vents and in thermal springs (Brierley 1982) and may play a role in the leaching of ores. Similarly,

the filamentous, mesophilic bacteria *Thiothrix* and *Beggiotoa*, which oxidize sulfide to elemental sulfur, have potential for ore beneficiation.

The Commercial Leaching Operation

The release and recovery of metals from ores are facilitated by methods designed to amplify the requisite oxidation-reduction reactions (Brierley 1978, Brierley 1982, Campbell et al. 1985).

Dump leaching: This operation is usually applied to the extraction of copper from low-grade oxide or sulfide ores which are hauled as uncrushed stones from open-pit mines to enormous dumps (see fig. 30). The dumps are sprinkled with water and the percolate is collected in a natural or artificial catch basin. The copper is removed from the acidic leachate by cementation (with iron), electrolysis, or solvent extraction; the solution is then recycled through the dump. The operation continues for years during which time sulfur- and metal-oxidizing bacteria grow extensively to perpetuate the leaching process.



Cementation

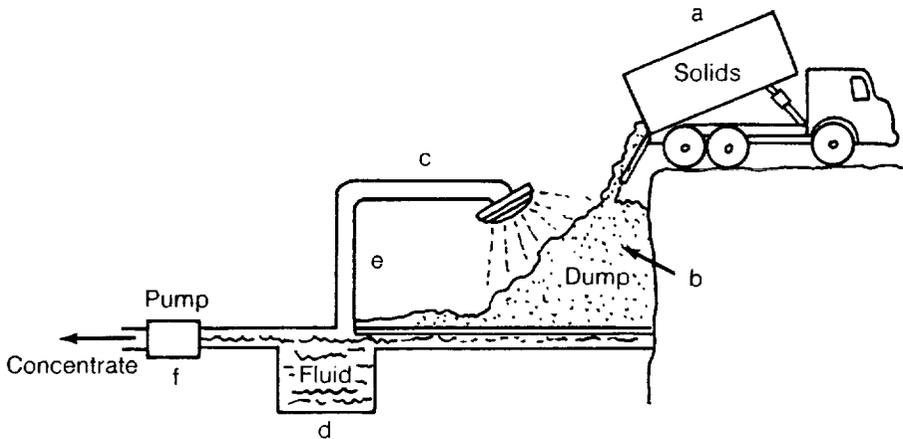


Figure 30

Dump Leaching

Uncrushed stones of low-grade oxide or sulfide ores (a) are hauled from the mining pit to a dump (b), where they are sprayed with a water solution (c). The influent solution, aided by the growth of sulfur- and metal-oxidizing bacteria, leaches metal salts from the ore. The salt-rich leachate is collected in an effluent pond (d) and either recycled immediately (e) to pick up more of the metal-containing salts or pumped (f) to the processing plant for removal of the metal.

Heap leaching: This operation is identical in principle to the dump process but employs crushed, medium-grade ores piled on an impermeable basin from which the leachate is recovered, the solute is removed from it, and the

leachate is concentrated and recycled (see fig. 31). This leaching operation is usually completed in a matter of months. To encourage the desired aerobic state, aeration tunnels are generally installed.

Figure 31

Heap Leaching Operation at the Gold Quarry Mine in Northeast Nevada

In this operation, a solution containing 125 ppm sodium cyanide is sprayed on the ore heap. The solution percolates down through the ore, dissolving gold from it. The gold-enriched percolate is then trapped by a plastic sheet, which channels the solution to a collecting pond, where it is pumped to the plant for removal of the gold and recycling of the solution.

The sodium cyanide heap leaching process is used on ores having an average gold concentration of 0.03 ounce/ton (0.9 ppm) and is economically attractive down to 0.01 ounce/ton (0.3 ppm). The mine shown is the largest gold leaching operation in the world.

This process does not use bacteria. However, somewhat analogous processes use bacteria in acidic solutions to extract copper from sulfide ores in heap or dump operations.

Courtesy of Komar Kawatra, Michigan Technological University, and Leonard Kroc, Newmont Gold Company



Vat leaching: Vat leaching is a purely chemical process by which concentrates of copper oxide ores are extracted by agitation with measured volumes of sulfuric acid. Research indicates that sulfidic ores can be extracted economically by the vat process using bacterial leaching (Brierley 1982, Torma and Banhegyi 1984).

In situ leaching: In selected mining sites that are inaccessible or abandoned because the high-grade ore has been recovered, acid leaching solutions are applied directly to either shallow or deep deposits and the leachates subsequently collected via wells. The process is confined to copper and uranium ores. Naturally occurring bacteria may augment this chemical process. It has been speculated that populations of appropriate thiobacilli and other sulfur-oxidizing bacteria could be injected into the locus to hasten and enhance the extraction. However, such a method is beset with difficulties, largely because the pathway of percolation is impossible to predict or control, thus endangering the quality of ground and surface waters. On the waterless Moon, the pollution of ground waters would not be a problem, but the extravagant use of water transported from Earth would be prohibitive.

In my opinion the only beneficiation operation having any potential for application on the Moon, or other

space body, would be microbe-enhanced vat leaching, inasmuch as the bacteria must be provided with a confined, minimally sized Earth-like environment, as I have indicated. The oxygen for the bioprocessing unit would come from the reductive chemical processing of ilmenite, or other oxide ore, via electrolyzed water resulting from the reaction. Thus, chemical processing of lunar ores (or some other local means of producing oxygen) must precede any biobeneficiation for it to be economical. It seems unlikely that bioprocessing of ores would ever become a part of a closed or semi-closed regenerative ecological system unless the chemical beneficiation process proved to be ineffective (an unlikely prospect) and oxygen from eucaryotic photosynthesis could be spared for the bacterial processing of ores. Most likely, if cost-benefit analysis indicated any virtue to bioprocessing, the operation would be conducted outside of any human settlement and tended either by robots or humans in suitable "space suits."

Other Microbial Transformations of Metals

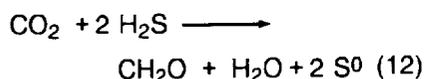
Anaerobic (Reducing) Conditions

In the absence of molecular oxygen, anaerobic or facultatively anaerobic bacteria are able to

reduce sulfur or metals leading to the formation of sulfidic ores and reduced forms of iron, copper, and other metals.

Sulfate-reducing bacteria: Two large groups of chemosynthetic, heterotrophic bacteria are able to oxidize certain organic acids and utilize sulfate, sulfur in other oxidized states, and elemental sulfur as electron acceptors. These bacteria, classified in such genera as *Desulfovibrio*, *Desulfotomaculum*, and *Desulfuromonas*, are found in anaerobic aquatic habitats containing the various oxidized states of sulfur, which are reduced to hydrogen sulfide, thus leading to the deposition of sulfidic ores.

Photosynthetic bacteria: Among the photosynthetic bacteria are two groups that utilize hydrogen sulfide under anaerobic conditions as a source of electrons in reducing carbon dioxide:



In one group, the "purple sulfur bacteria," the sulfur is deposited intracellularly. In another group, the "green sulfur bacteria," the sulfur is deposited outside the bacterial cell and subsequently oxidized to sulfate in the presence of hydrogen gas (source of electrons). These, and other photosynthetic bacteria, are

anaerobic and thus unable to utilize oxygen as the final electron acceptor. The purple sulfur bacteria comprise ten genera (e.g., *Thiospirillum*, *Thiocystis*, and *Amoebobacter*); the green sulfur bacteria comprise four genera (e.g., *Chlorobium* and *Pelodictyon*).

Bacterial reduction of metal ions: Some strains of members of the genera *Thiobacillus* and *Sulfolobus* are able to reduce Fe^{+++} to Fe^{++} , obtaining reducing power from elemental or reduced sulfur, which is oxidized in the process. A number of other soil bacteria are able to reduce ferric iron as well. Manganese is both reduced and oxidized by various marine and soil bacteria. Ferromanganese nodules found in the oceans are laid down slowly through the bacterial reduction and oxidation of ferric and manganese ions (Summers and Silver 1978, Ehrlich 1981, Ehrlich and Holmes 1986).

Biotransformations of Some Toxic Metals and Metalloids

Within any land or aquatic ecosystem, toxic and nontoxic cations, whether naturally or artificially introduced, may be absorbed and metabolized by certain species among the indigenous microflora. In the process, inherent toxicities are often neutralized or modified. An excellent case in point is mercury which is highly toxic (binds

sulfhydryl groups) as Hg^{++} but is enzymatically reduced by various mercury-resistant microorganisms to its volatile and nontoxic state as Hg^0 (Belliveau et al. 1987, Decker 1986, Hutchins et al. 1986, Thompson 1986).

Likewise certain toxic metals are methylated through the action of methylcobalamin excreted by a number of different aquatic and soil bacteria. This methylation does not necessarily detoxify the metal. In the case of mercury, the methyl and dimethyl forms can subsequently be concentrated in certain organisms, especially fish. Other metals that can be methylated by bacteria include tin, cadmium, lead, and arsenic.

Some bacteria can cleave certain organic mercurials into Hg^{++} plus an organic residue. Arsenic as As^{3+} can be oxidized by a number of bacteria to As^{5+} ; in either state of oxidation, arsenic is toxic, but far less so in the pentavalent state. Reduced arsenic reacts with sulfhydryl groups; oxidized arsenic simulates phosphate in metabolic pathways. As oxoanions, tellurium and selenium are toxic to many bacteria. Some bacteria are able to reduce these salts to Te^0 or Se^0 , thereby destroying their toxicity.

Since lunar rock contains virtually the same spectrum of toxic metals as found on the Earth (Morris et al. 1983), the biobeneficiation of ores on the Moon could be

compromised by the presence of toxic cations in ore slurries, unless the bacteria introduced into the vats are resistant to high levels of cations of the more abundant toxic metals, including those to be recovered. Resistance to toxic metals is genetically endowed and can be introduced through gene recombination techniques in at least some of the bacterial species involved in the beneficiation of ores or in the removal of toxic cations from an aquatic environment (Belliveau et al. 1987, Ehrlich and Holmes 1986, Hughes and Poole 1989, Hutchins et al. 1986, Nicolaidis 1987, Pooley 1982, Torma 1987a, Trevors 1987, Tsezos 1985, Woods and Rawlings 1985).

Leaching of Ores by Growth of Heterotrophic Microorganisms

Sometimes sufficient organic matter is found in ores to support the growth of diverse microbes, resulting in the production of organic acids (e.g., lactic, citric, acetic, glutamic, and glycolic) which lower the pH, helping to solubilize the metals and encouraging the development of an acidophilic microflora of thiobacilli. This may be viewed as a synergistic effect.

On the other hand, alkaline leaching may be encouraged in the presence of organic nitrogen

(protein, amino acids, purines, pyrimidines, etc.) as a result of deaminations catalyzed by heterotrophs, yielding ammonia which, in aqueous solution, becomes ammonium hydroxide. Some metals, notably copper, cobalt, and zinc, are compounded by ammonium hydroxide.

Calculations suggest it is not feasible to harness heterotrophs for the leaching of ores. Enormous quantities of decomposable organic matter would need to be provided in order for either acid or alkaline leaching to function at a commercial level (Kelly, Norris, and Brierley 1979). The use of organic wastes from sewage treatment plants has been investigated (Hutchins et al. 1986).

Bioaccumulation of Metals

Some microorganisms are capable of assimilating large amounts of metals from solution. The outlook for exploitation of such organisms for the removal of toxic ions or for the concentration of useful metals is very bright indeed (Ehrlich and Holmes 1986, Hughes and Poole 1989, Torma 1987a and b, Volesky 1987). Of course, all living creatures require certain trace elements which are found in low concentrations within all cells, though they would be toxic in

higher concentrations. Certain microorganisms, however, are endowed with the capacity to assimilate large amounts of certain metals, even toxic ones.

Electrostatic Attraction

A variety of functional groups, or ligands, on cell surfaces carry positive or negative charges, conveying a net charge to the cell. Other things being equal, the intensity and sign (+ or -) of the charge depends on the pH of the extracellular environment. In most natural environments the pH is higher than the cell's isoelectric point; therefore, the cell will have a net negative charge and will passively attract cations, much like an ion exchange resin.

However, there is some selectivity, suggesting the existence of specific binding sites for particular cations on the various surface structures. Certain fungi and bacteria, for example, bind large quantities of uranium ions, in some instances to the extent of 15 percent of the cells' dry weight. A yeast was found to bind mercury to its cell walls in amounts equivalent to the weight of the cell walls themselves. Certain species of algae and fungi concentrate copper to the extent of 12 percent of the cells' dry weight. Other metals bound by electrostatic attraction include Fe, La, Cd, Ca, Mg, Pb, Ni, Mn, Zn, Ag, K, and Na.

Surface Deposition or Precipitation (Biosorption)

Massive amounts of metals or insoluble metal compounds can be deposited on the surfaces of some microorganisms. This deposition will occur in some instances when the metal is metabolized; in other instances no transformation of the metal is required for its deposition (Belliveau et al. 1987, Ehrlich and Holmes 1986, Olson and Kelly 1986, Thompson 1986).

Those species of bacteria that reduce tellurite or selenite to metallic Te or Se deposit the metals on their surfaces, accumulate them intracellularly, or both. Some bacteria will aggregate insoluble lead compounds on their surfaces. Most common is the precipitation of ferric compounds and manganic oxides. While many bacteria can oxidize the manganous ion, sheathed, filamentous bacteria in the genera *Hyphomicrobium* and *Metallogenium* and in the *Sphaerotilus-Leptothrix* group become heavily coated with manganic oxides. Also, *Sphaerotilus-Leptothrix* and a group of stalked bacteria in the genus *Gallionella* acquire heavy deposits of oxidized iron, largely ferric hydroxide. Apparently, gallionellae derive energy through the oxidation of Fe^{+2} , while the sheathed, filamentous group

merely attract the insoluble ferric hydroxide to their sheaths. The genus *Zoogloea*, which is common in activated sludge sewage operation systems, produces copious quantities of polysaccharide slime having high affinity for Cu^{+2} , Cd^{+2} , Co^{+2} , Ni^{+2} , Zn^{+2} , and Fe^{+3} (Hutchins et al. 1986). The production of extracellular polysaccharide slimes is common to many bacteria; chemically, they vary considerably from species to species, even from strain to strain (Ehrlich and Holmes 1986, Thompson 1986, Torma 1987a, Volesky 1987).

Intracellular Transport of Ions

Both monovalent (Na^{+} , K^{+} , Cs^{+} , Li^{+} , Tl^{+} , and NH_4^{+}) and divalent cations are specifically transported to satisfy nutritional needs of microorganisms. In some cases, rather high intracellular concentrations of certain metals are achieved. Often, the same transport mechanism will function for several cations; e.g., Mg^{+2} , Co^{+2} , Mn^{+2} , Ni^{+2} , and Zn^{+2} in *Escherichia coli*. Others are more specific, although in all such systems various other cations will compete with a particular cation for uptake, in some cases even inducing efflux of ions. Fungi appear to concentrate metals or metalloids to a somewhat greater extent than do most bacteria (Hutchins et al. 1986, Summers and Silver 1978, Tsezos 1985).

Many of the metal ions are toxic, although the toxicity varies considerably from species to species (Belliveau et al. 1987, Ehrlich 1981, Summers and Silver 1978). Sometimes the inhibition of bacterial growth is synergistic. For example, *Klebsiella aerogenes* has been shown to be inhibited to a greater extent by Cd and Zn than by the sum of the individual toxicities of the two cations. The presence of clays, certain anions, and organic matter of various kinds often markedly reduces metal toxicity. Not surprisingly, metal chelators (e.g., ethylenediamine tetraacetic acid and citric acid) suppress the toxicity of many cations toward microorganisms. Clearly, toxicity is an important consideration in harnessing microbial cells to concentrate metals or metalloids.

An interesting case is found with the diatoms, which encase themselves in siliceous shells in an amazing variety of beautiful shapes. The uptake of silicates by diatoms has been shown to be competitively inhibited by germanic acid, thereby suggesting a means of recovering germanium from natural sources (Kelly, Norris, and Brierley 1979; Krumbein 1983). (See below for another aspect of silicate uptake.) Incidentally, the Russians for over 20 years have referred to "silicate bacteria," which they claim, in English abstracts, will free silicates from aluminosilicate ores, thereby

beneficiating aluminum oxide (Alexandrov, Ternovskaya, and Blagodyr 1967; Andreev, Lycheva, and Segodina 1979; Andreev, Pol'kin, and Lyubarskaya 1982; Rohatgi, Trivedi, and Rohatgi 1984).

Not to be overlooked is the uptake of oxoanions, especially sulfate, the transport of which has been shown in *Salmonella typhimurium* to be competitively inhibited by chromate, selenite, molybdate, tungstate, and vanodate (descending order of effectiveness).

Natural Chelators

Some naturally occurring, low molecular weight compounds (citric acid, aspartic acid, and a number of dicarboxylic acids) have long been known to chelate various metal ions. Many microorganisms produce organic compounds that can do the same thing. Such organic compounds are collectively called "ionophores," the best known of which are siderophores (iron-attractors) (Nielands 1981; Brock, Smith, and Madigan 1984). Microbes that produce siderophores are believed to have evolved as the rising oxygen content of the Earth's atmosphere caused oxidation of much of the iron into its insoluble oxide and hydroxide states. To capture the minute quantities of free ferric ions that exist, microbes capable of synthesizing chelators of ferric iron arose. Incidentally, animals

(including humans) trap iron through iron-binding proteins such as lactoferrin, transferrin, and ferridoxin, the latter being particularly abundant in the liver. Many pathogenic bacteria and fungi compete with the infected host for iron through the formation of siderophores. The siderophores—phenolates, hydroxamates, or cyclic peptides—are capable of binding ferric iron, which is subsequently transported into the cell, released, reduced enzymatically, and secreted back into the environment for recycling.

Recently, an ionophore for silicate, another ion very sparingly available, has been found in diatoms (Bhattacharyya and Volcani 1983).

Other metal-binding organic molecules, not chelators, abound in living systems. Of particular note is metallothionein, which binds a variety of cations by virtue of its available sulfhydryl (-SH) groups (Kägi and Kojima 1987).

Conclusion

As I emphasized in my introduction, the rigors of the Moon, or other space environments (asteroids, Mars, Phobos, Deimos, a station orbiting the Earth) are inimical to terrestrial life forms, including, of course, microorganisms. While it would be a far simpler matter to provide, on the Moon for example,

conditions conducive to microbial existence than those conducive to human life, no ore could be benefited by bacteria there without the provision of a gas-tight container affording certain minimal conditions. No doubt the partial pressures of O₂, N₂, and CO₂ could be held to levels substantially lower than those found on the Earth—how much lower would have to be determined experimentally. The water supply would need to be adequate and continuous. Full radiation protection would be necessary, and temperature fluctuations would need to be minimized. It might well be best to select thermophilic bacteria for this endeavor.

Once a biobeneficiation reactor was constructed and all support systems were activated, it would be inoculated with appropriate strains of bacteria. More than likely, lyophilized (freeze-dried) cultures, probably of genetically engineered strains, would be reconstituted onsite in an aqueous solution, containing a mixture of nutrients brought from Earth as a dehydrated powder. The culture would be added to the moistened ore bed once the operator was satisfied that the cells were growing within the culture vessel. Initially, a leaching solution of dilute sulfuric acid would be added. The enclosure would have to be tight enough to retain all water vapors as well as the atmospheric gases.

As the size of the operation increased by expanding the size of the incubator, more water would need to be added. Unless sufficient sulfur or reduced sulfur in the ore was available for biological oxidation, additional sulfuric acid would be required. As the bacteria became established (as measured by the growth of a subculture or by microscopic examination of samples from the reactor or by chemical determination of the ratio of the concentrations of Fe^{+++} and Fe^{++}), further additions of the culture would become unnecessary. Before the biological operation was established, chemical reductive processing of oxide ores (e.g., ilmenite) would need to be functioning well to provide the necessary water and oxygen.

One problem with the foregoing scenario is that some of the minerals or elements in the ore might be toxic to the bacteria. Studies conducted more than 15 years ago revealed that lunar fines or their extracts inhibited as well as stimulated or proved innocuous to a variety of microorganisms (Silverman, Munoz, and Oyama 1971; Taylor et al. 1970; Taylor et al. 1971; Taylor and Wooley 1973). Silverman and colleagues found that, while the lunar substrate stimulated pigment production by the bacterium *Pseudomonas aeruginosa*, it inhibited pigment

production by the bacterium *Serratia marcescens*. Others (Walkinshaw et al. 1970, Walkinshaw and Johnson 1971) found that lunar soil enhanced chlorophyll production and early development of ferns, bryophytes, and a number of seed plants. In keeping with these pioneering studies of the interplay between lunar materials and living organisms, I undertook to make a preliminary study (Johansson 1984) of the effects of leachates of lunar fines on the growth of the common colon bacillus, *Escherichia coli*, in a glucose medium with minimal mineral salts. I found that, depending on its concentration, the leachate either enhanced or inhibited the growth of the bacteria. The specific elements or minerals responsible for the various biological responses to the lunar materials have not been identified.

Prior terrestrial research would be necessary to provide guidance on dealing with a toxicity problem, if it could be identified in advance by appropriate tests of lunar materials. Means of dealing with such a problem could include the addition of hydrogen sulfide, chelators, or certain mineral salts known to block specific toxic ions. There is a vast literature on handling toxicity, but research specifically applied to lunar or asteroidal materials is needed.

Another biological approach to the problem of recovery of certain metals from lunar ores might be the application of metal-binding agents produced, at least initially, on Earth. Candidate substances include ionophores and metallothionein.

The introduction of the micro-organisms needed for the bioprocessing of lunar ores would probably not be an early event in the establishment of an outpost on

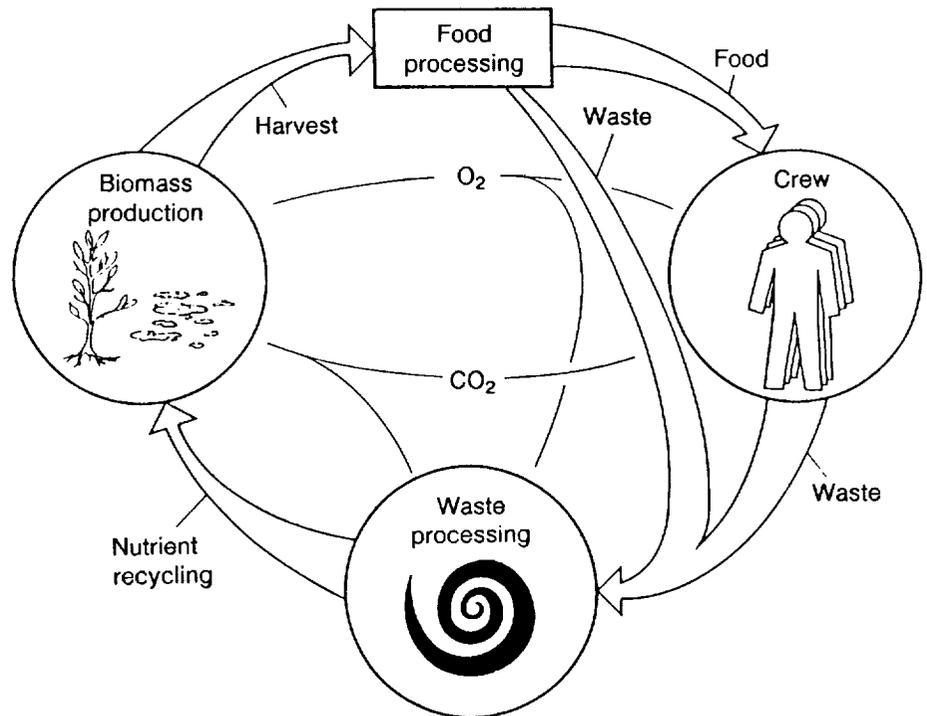
the Moon. If a human habitation with recycling (of water, oxygen, carbon dioxide, nitrogen, sulfur, and other essential nutrients) was established, the cultures needed in the mining operation would be useful as part of the microflora of that ecosystem. The thiobacilli and other bacteria involved in the leaching of ores are important in the cycles of sulfur, nitrogen (some fix N_2), carbon, and oxygen on Earth (see fig. 32).

Figure 32

Regenerative Life Support System

In a controlled ecological life support system, as diagramed here, biological and physicochemical subsystems would produce plants for food and process solid, liquid, and gaseous wastes for reuse in the system.

A bioprocessing unit, in which bacteria oxidize and catalyze the extraction of metals from their lunar or asteroidal ores, could be incorporated into this regenerative system. The bioprocessing unit would contribute to the gas and nutrient recycling, the biomass inventory, and the waste processing of the larger regenerative life support system.



One concern is the availability in the lunar soil of the sulfur needed for the ore-beneficiating bacteria to gain energy and by the same process to produce the sulfate for the acid leaching process. In this regard, carbonaceous chondritic asteroids might yield material more suitable for biological ore beneficiation than would the Moon. Of course, sulfur from the Earth could be added to the beneficiation enclosure on the Moon. A fringe benefit of using asteroidal material would be its content of organic matter, which desirable heterotrophic bacteria might possibly utilize for their carbon needs.

Another question that needs to be answered is whether the low level of nitrogen in the lunar regolith (Gibson 1975) is sufficient to enable significant bacterial colonization. It may be necessary to provide, at the onset, nitrogen in the form of nitrate. (Ammonium nitrogen would work but some of it would be oxidized to nitrate, thus imposing a demand on atmospheric oxygen.) The amount of phosphorus may also not be adequate for bacterial life on the Moon. These bacterial nutritional problems would also apply to the development of an ecosystem supporting human life.

In summary, bioprocessing using bacteria in closed reactors may be a viable option for the recovery of

metals from the lunar regolith. Obviously, considerable research must be done to define the process, specify the appropriate bacteria, determine the necessary conditions and limitations, and evaluate the overall feasibility.

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